

AMENDMENT

IN THE CLAIMS:

Please cancel claims 33-38 and 40-41 without prejudice as drawn to a non-elected invention. Please cancel claim 1 without prejudice or disclaimer. Please amend claims 2-5, 8, 12, 15-18, and 21-22 and add new claims 42-45 pursuant to 37 C.F.R. §1.121 as follows (*see* the accompanying "marked up" version pursuant to 37 C.F.R. §1.121):

1. Canceled

2. (Amended) The expression plasmid of claim 42 wherein the pol I promoter is proximal to the polyadenylation signal and the pol I terminator sequence is proximal to the pol II promoter.

3. (Amended) The expression plasmid of claim 42 wherein the pol I promoter is proximal to the pol II promoter and the pol I terminator sequence is proximal to the polyadenylation signal.

4. (Amended) The expression plasmid of claim 42 wherein the plasmid has a map selected from the group consisting of pHW2000, pHW11 and pHW12.

5. (Amended) An expression plasmid comprising viral cDNA corresponding to a genomic segment of a segmented negative strand RNA virus, wherein the cDNA is inserted between an RNA polymerase I (pol I) promoter and a regulatory element for the synthesis of vRNA or cRNA with the exact 3' end, which are in turn inserted between an RNA polymerase II (pol II) promoter and a polyadenylation signal.

6. (Unchanged) The expression plasmid of claim 5, wherein the negative strand RNA virus is a member of the *Orthomyxoviridae* virus family.

7. (Unchanged) The expression plasmid of claim 6, wherein the virus is an influenza A virus.

8. (Amended) The expression plasmid of claim 7, wherein the influenza viral genomic segment (i) encodes a protein selected from the group consisting of a viral polymerase complex protein, M protein, and NS protein; and (ii) is derived from a strain well adapted to grow in cell culture or from an attenuated strain, or both.

9. (Unchanged) The expression plasmid of claim 6, wherein the virus is an influenza B virus.

10. (Unchanged) The expression plasmid of claim 8 wherein the plasmid has a map selected from the group consisting of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW245-NP, pHW247-M, and pHW248-NS.

11. (Unchanged) The expression plasmid of claim 8 wherein the plasmid has a map selected from the group consisting of pHW181-PB2, pHW182-PB1, pHW183-PA, pHW185-NP, pHW187-M, and pHW188-NS.

B3 12. (Amended) The expression plasmid of claim 7, wherein the influenza viral genomic segment comprises a gene selected from the group consisting of a hemagglutinin (HA) gene and a neuraminidase (NA) gene.

13. (Unchanged) The expression plasmid of claim 12, wherein the influenza gene is from a pathogenic influenza virus strain.

14. (Unchanged) The expression plasmid of claim 12, wherein the plasmid has a map selected from the group consisting of pHW244-HA, pHW246-NA, pHW184-HA, and pHW186-NA.

15. (Amended) A minimum plasmid-based system for the generation of infectious segmented negative strand RNA viruses from cloned viral cDNA comprising a set of plasmids wherein each plasmid comprises one viral genomic segment, and wherein the viral cDNA corresponding to the viral genomic segment is inserted between an RNA polymerase I (pol I) promoter and a regulatory element for the synthesis of vRNA or cRNA with the exact 3' end, thereby resulting in expression of vRNA or cRNA, which are in turn inserted between an RNA polymerase II (pol II) promoter and a polyadenylation signal, thereby resulting in expression of viral mRNA.

16. (Amended) The minimum plasmid-based system of claim 44, wherein the pol I promoter is proximal to the polyadenylation signal and the pol I terminator sequence is proximal to the pol II promoter.

17. (Amended) The minimum plasmid-based system of claim 44, wherein the pol I promoter is proximal to the pol II promoter and the pol I terminator sequence is proximal to the polyadenylation signal.

18. (Amended) The minimum plasmid-based system of claim 15, wherein the negative strand RNA virus is a member of the *Orthomyxoviridae* virus family.

19. (Unchanged) The plasmid-based system of claim 18, wherein the virus is an influenza A virus.

20. (Unchanged) The plasmid-based system of claim 18, wherein the virus is an influenza B virus.

21. (Amended) The plasmid-based system of claim 19, wherein the viral genomic segment (i) encodes a protein selected from the group consisting of a viral polymerase complex protein, M protein and NS protein; and (ii) is derived from a strain well adapted to grow in cell culture or from an attenuated strain, or both.

B5 22. (Amended) The plasmid-based system of claim 19, wherein the viral genomic segment comprises hemagglutinin (HA) gene, or neuraminidase (NA) gene, or both; wherein said genes are from a pathogenic influenza virus.

23. (Unchanged) The plasmid-based system of claim 19 wherein said system comprises one or more plasmids having a map selected from the group consisting of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and pHW248-NS.

24. (Unchanged) The plasmid-based system of claim 19, wherein said system comprises one or more plasmids having a map selected from the group consisting of pHW181-PB2, pHW182-PB1, pHW183-PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M, and pHW188-NS.

25. (Unchanged) A host cell comprising the plasmid-based system of claim 15.

26. (Unchanged) A host cell comprising the plasmid-based system of claim 18.
27. (Unchanged) A host cell comprising the plasmid-based system of claim 19.
28. (Unchanged) A host cell comprising the plasmid-based system of claim 22.
29. (Unchanged) A method for producing a negative strand RNA virus virion, which method comprises culturing the host cell of claim 25 under conditions that permit production of viral proteins and vRNA or cRNA.
30. (Unchanged) A method for producing an *Orthomyxoviridae* virion, which method comprises culturing the host cell of claim 26 under conditions that permit production of viral proteins and vRNA or cRNA.
31. (Unchanged) A method for producing an influenza virion, which method comprises culturing the host cell of claim 27 under conditions that permit production of viral proteins and vRNA or cRNA.
32. (Unchanged) A method for producing a pathogenic influenza virion, which method comprises culturing the host cell of claim 28 under conditions that permit production of viral proteins and vRNA or cRNA.
- 33-38. Canceled ✓

39. (Unchanged) A method for generating an attenuated negative strand RNA virus, which method comprises:

- (a) mutating one or more viral genes in the plasmid-based system of claim 15;
- and
- (b) determining whether infectious RNA viruses produced by the system are attenuated.

40-41. Canceled

42. (New) The expression plasmid of claim 5, wherein the regulatory element for the synthesis of vRNA or cRNA with the exact 3' end is an RNA polymerase I (pol I) terminator sequence.

43. (New) The expression plasmid of claim 5, wherein the regulatory element for the synthesis of vRNA or cRNA with the exact 3' end is a ribozyme sequence.

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44. (New) The minimum plasmid-based system of claim 15, wherein the regulatory element for the synthesis of vRNA or cRNA with the exact 3' end is an RNA polymerase I (pol I) terminator sequence.

45. (New) The minimum plasmid-based system of claim 15, wherein the regulatory element for the synthesis of vRNA or cRNA with the exact 3' end is a ribozyme sequence.
